

Nosema disease treatment with “Nozevit” – histology approach



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INTRODUCTION The Nosema disease is a parasitic disease of adult honey bees (*Apis mellifera*) caused by two described species of microsporidia, *Nosema apis* and *Nosema ceranae*, which in adverse living conditions forms spores. Honey bees afflicted with nosemosis start to forage earlier, while pathological changes of their mid – gut epithelial cells, as well as digestive and metabolic disorders, cause malnutrition and decrease of population size of honey bee colonies leading to premature deaths. Mucous cover formed by digestive tract mucosubstances acts in increasing slickness and protection from proteolytic degeneration and pathogen microorganisms, so we have predicted possibility that “Nozevit“ stimulate production higher amount of mucosa and in that way protect epithelial gut cells from *Nosema* sp. invasion.

MATERIAL AND METHODS The intestines of bees were fixed in a 4% formaldehyde solution, inserted in paraffin blocks and cut with a microtome to 6 – 7 μm thick sections. Dewaxed sections were stained for general morphological purposes according to Hemalaon-Eozinic method (HE) and for purpose of description of neutral glycoconjugates, acid and sulphate mucopolysaccharides and metachromasia we used Periodic Acid – Schiff Reaction (PAS) (Fig. 2) Alcian blue (pH = 2, 5), Alcian blue (pH = 1,0) and Toluidine blue (TB) specific staining (Fig. 4). For analysing stained microscopically preparations we used bright field microscope Olympus BX41 and have taken photographs with Olympus DP12 U – TVO camera.

RESULTS The results of general histological examination show that the lumen of treated bees is coated with a firm layer (Fig. 1a, 1b), while untreated bees have a much looser and not clearly limited area of peritrophic membrane. Also, it has been observed that the intestinal content with numerous *Nosema* spores tends to be squeezed in to the centre of intestine lumen, due to which germination of spores is probably impeded. „Nozevit“ treatment of diseased bees has laed to visable and significant increasing in AB (pH = 2,5) positive mucopolysaccharides (Fig.3), or glycoproteins with carboxyl groups, sialic acid or uranic acid and/or with sulphate esters. In the same time, amount of O - sulfated esters of mucopolysaccharides (AB, Ph = 1,0) (Fig. 5) in mucous coat of intestine wasn't show significant change in structure, in comparison with non treated bees. In diseased group treated with „Nozevit“ is clearly visable increasing amount of non sulfated mucosubstances in apper mucosa stratum.

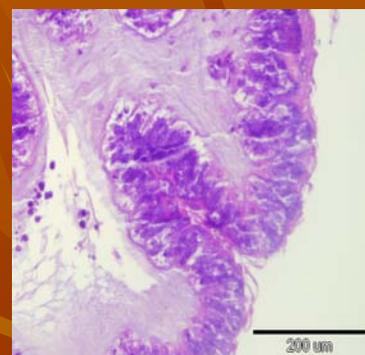
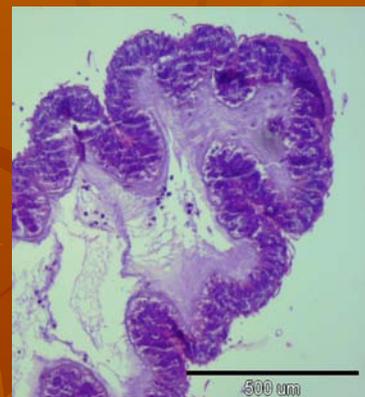


FIG: 1 a, 1 b

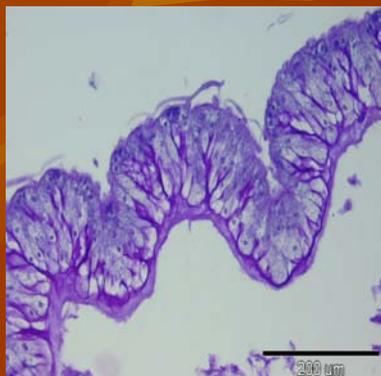


FIG. 2

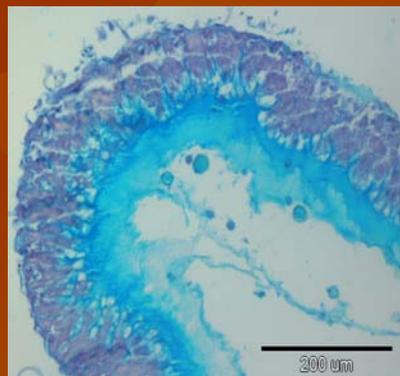


FIG. 3

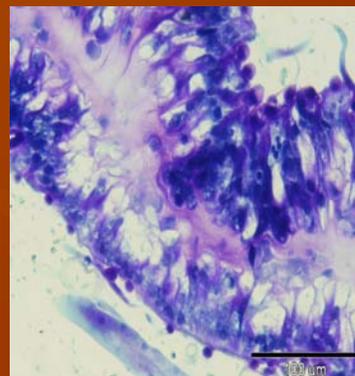


FIG. 4



FIG. 5

DISSCUSION AND CONCLUSION In this research histochemical analyses were show increasing in production glycoproteines with carboxylic groups and sialic acid - rich (AB, Ph = 2,5), and a large amount of TB methachromatic substances points to the presence of sialic acid – rich non sulfated mucopolysaccharides (TB) in superficial layer of intestine lumen in diseased bees after treatment with „Nozevit“. Functions of secreted mucous layer are lubricating undigested materials and osmoregulation, transfer of proteins or their fragments as well as of ions and fluids and protection from mechanical injuries or bacterial invasions. Mucosubstances secreted by the ventriculus (mid – gut) epithelium have been related to the absorbtion of easily digested moleculs and have very important role in intestinal absorbtion process. Based on presented datas we can conclude that herbal preparation „Nozevit“ induce production and secretion of mucous from epithelium layer and additionally stick to the mucosa to form a resilient membrane, and in same time ensure protection from new invasion with *Nosema* spores and course of normal physiological processes.